Strategies to Enhance Effects of Cellular Immunotherapy

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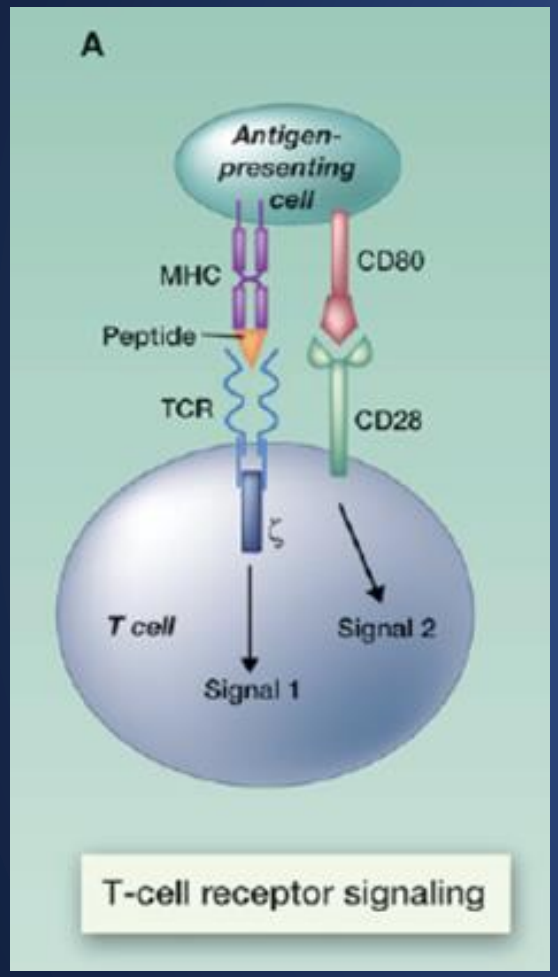
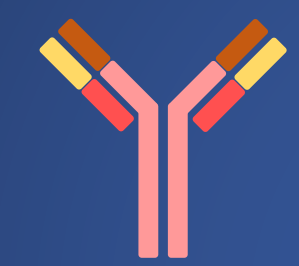
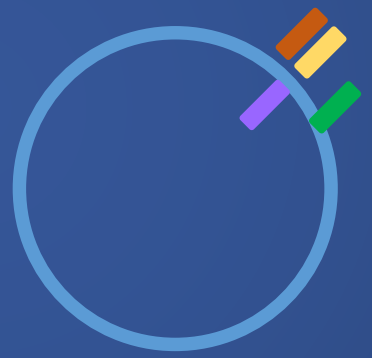
What is Chimeric antigen receptor (CAR) T cell therapy?





- Target antigens present on cancer cells
  - CD19
  - CD22
  - CD33

CAR T-cell

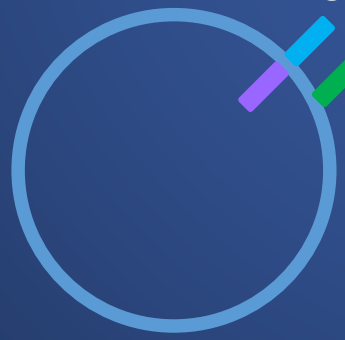


+

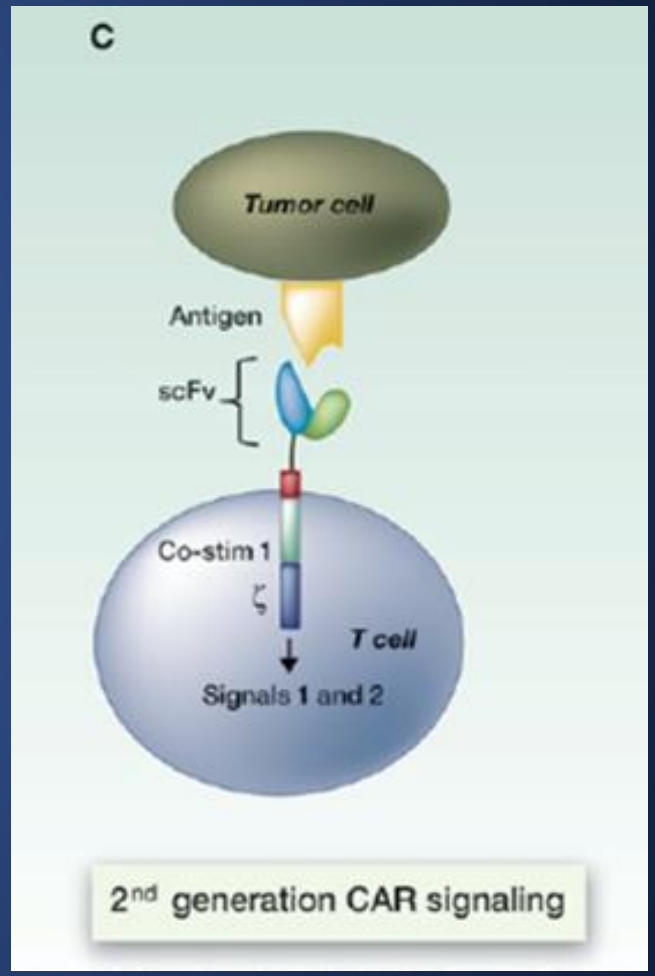
T-cell

TCR

CD28



- Potent killing

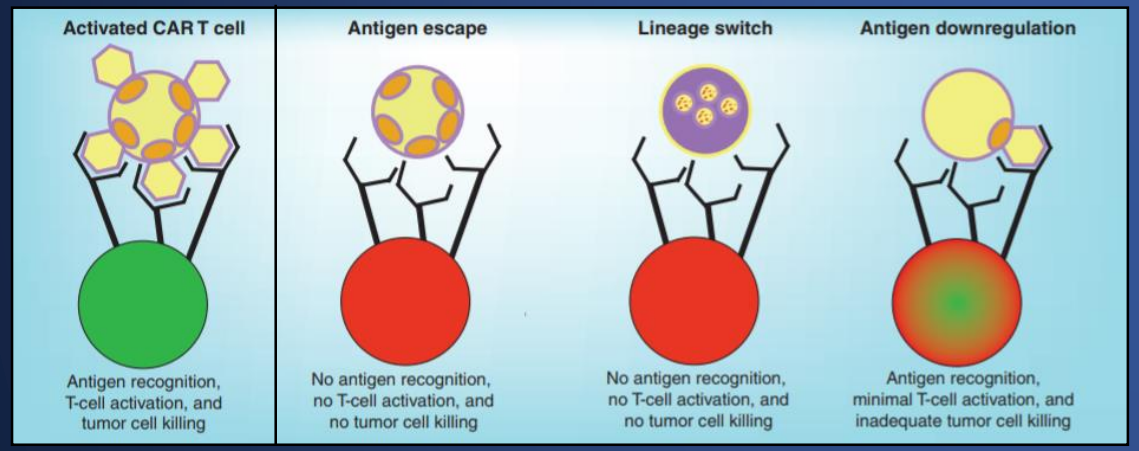


Lee, Clin Cancer Res 2012

How effective is CD19 CAR T-cell therapy in refractory pediatric B-ALL?

- 60-90% initial remission rate[1-4]
- 29-45% long-term relapse rate[5]

General mechanisms of disease resistance:



Adapted from (5)

Table 1. A summary of antigen escape in CD19 CAR trials for ALL

Trial	Relapse rate	CD19-negative relapse rate
Children's Hospital of Philadelphia phase I	36% (20/55)	24% (13/55)
Novartis phase II (ELIANA)	33% (20/61)	25% (15/61)
Seattle Children's Research Institute phase I	45% (18/40)	18% (7/40)
NCI phase I	29% (8/28)	18% (5/28)
Memorial Sloan Kettering phase I	57% (25/44)	9% (4/44)
Fred Hutchinson Cancer Center phase I	31% (9/29)	7% (2/29)

Adapted from (5)

Mechanisms of resistance to CD19 CAR:

- CD19 genetic mutations and loss of heterozygosity[6]
- Splicing variants[7]
- Loss of CD81[8]
- Lineage switch[9]

1. Lee DW et. al, Lancet 2015; 2. Davila ML et. al., Sci Transl Med 2014; 3.Maude SL New Engl J Med 2014; 4. Kochenderfer JN J Clin Oncol 2014 ;
5. Majzner, RG and Mackall, CL Cancer Discov 2018
6. Orlando, et. Al. Nat. Med. (2018); 7. Sotillo, E. Et. Al. Cancer Discov (2015); 8. Braig F et. Al. Blood (2017) 9. Jacoby, E. Et. Al, Nat. Commun (2016)

Is there still a role for the cancer biologist in the era of CAR T cell therapy?

Can we change target expression to increase the efficacy of CAR-targeting therapies?

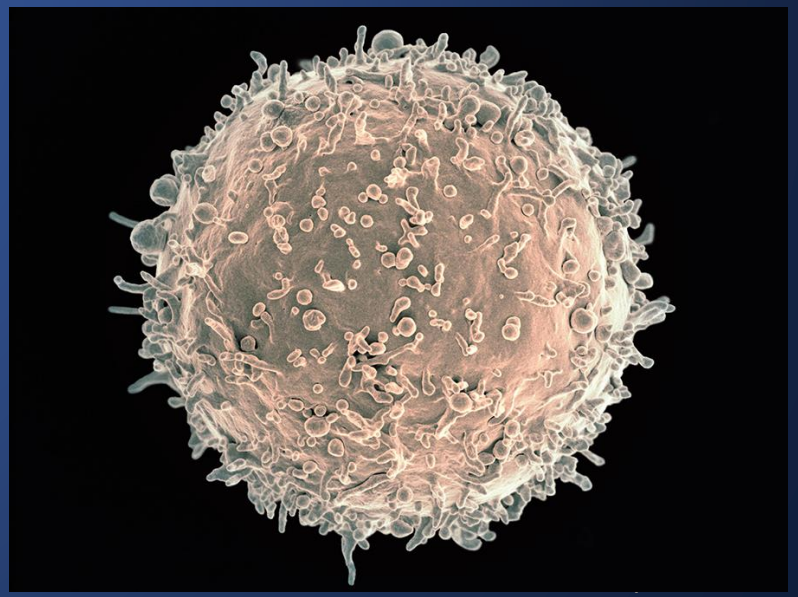
Small molecule screen for molecules that alter the antigens presentation on the cancer cell

Can we identify new antigenic targets?

Antigen mapping and new antigen target discovery

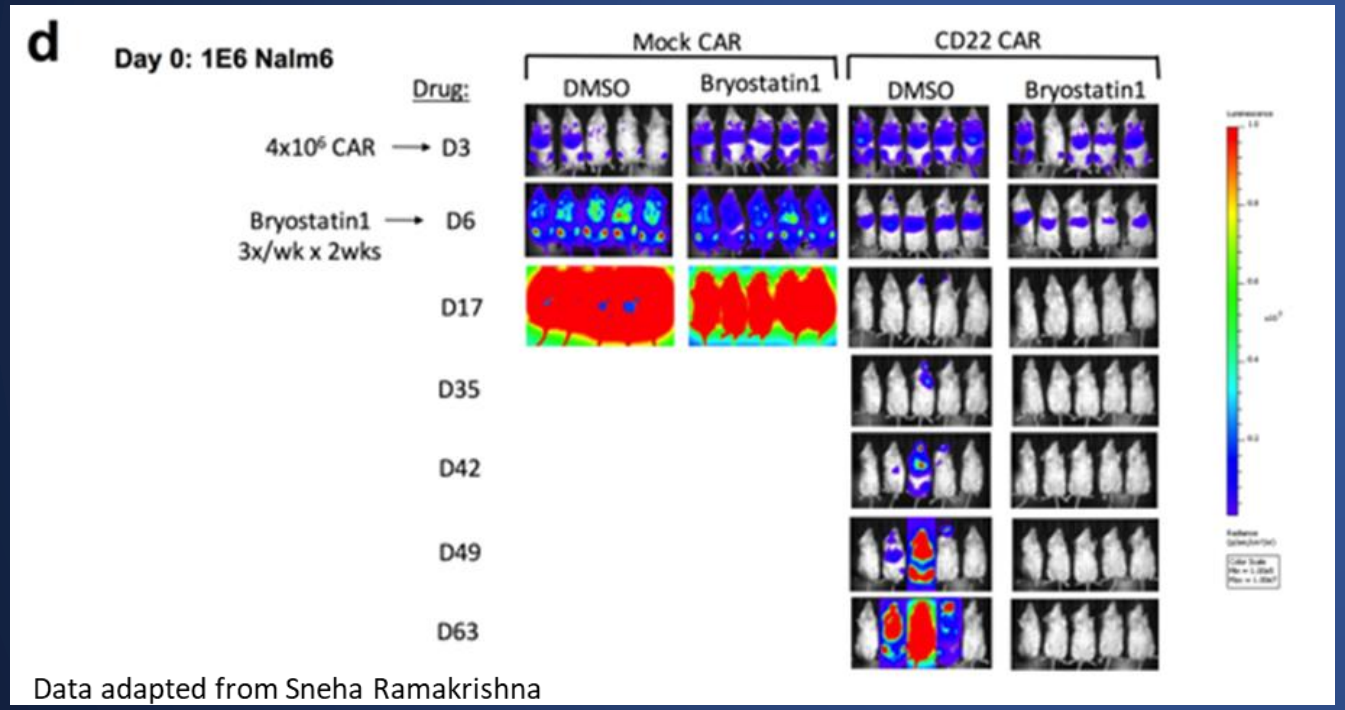
- Surfaceome MS + high coverage total RNAseq

  (Collaboration with Richard Aplenc – CHOP)



Can we change target expression to increase the efficacy of CAR-targeting therapies?

Increasing target expression with bryostatin1 improves CD22 CAR function:



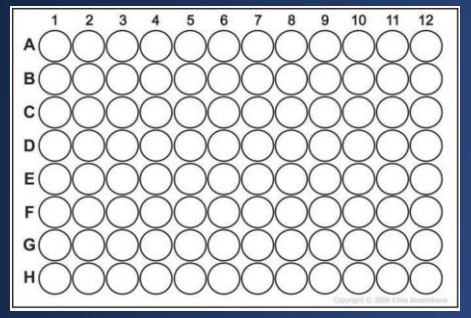
Data adapted from Sneha Ramakrishna

Clin Cancer Res. 2019 May 20.

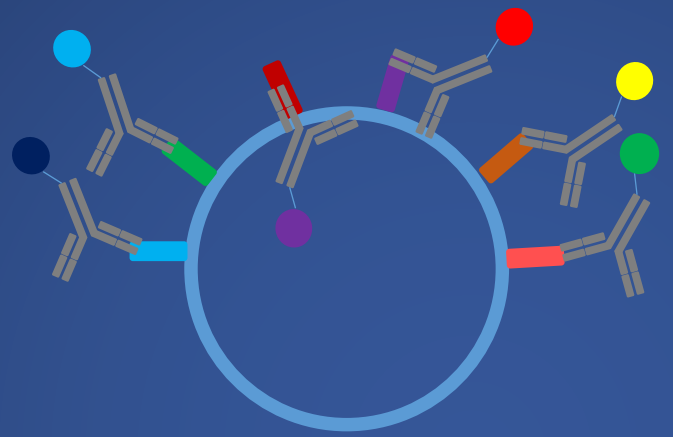
Project goals:

- Primary goal: Identify drugs that increase CD19 surface expression
- Additional goals:
  1. Identify drugs that affect other CAR targets (CD22, TSLPR, CD33, FLT3)
  2. Generate mechanistic data on CD19 regulation
  3. Analyze the clinical relevance of drugs that change CAR target expression

Design of a screen for drugs that affect antigen expression



- 96 well format with tiled drugs
- Leukemia cell line



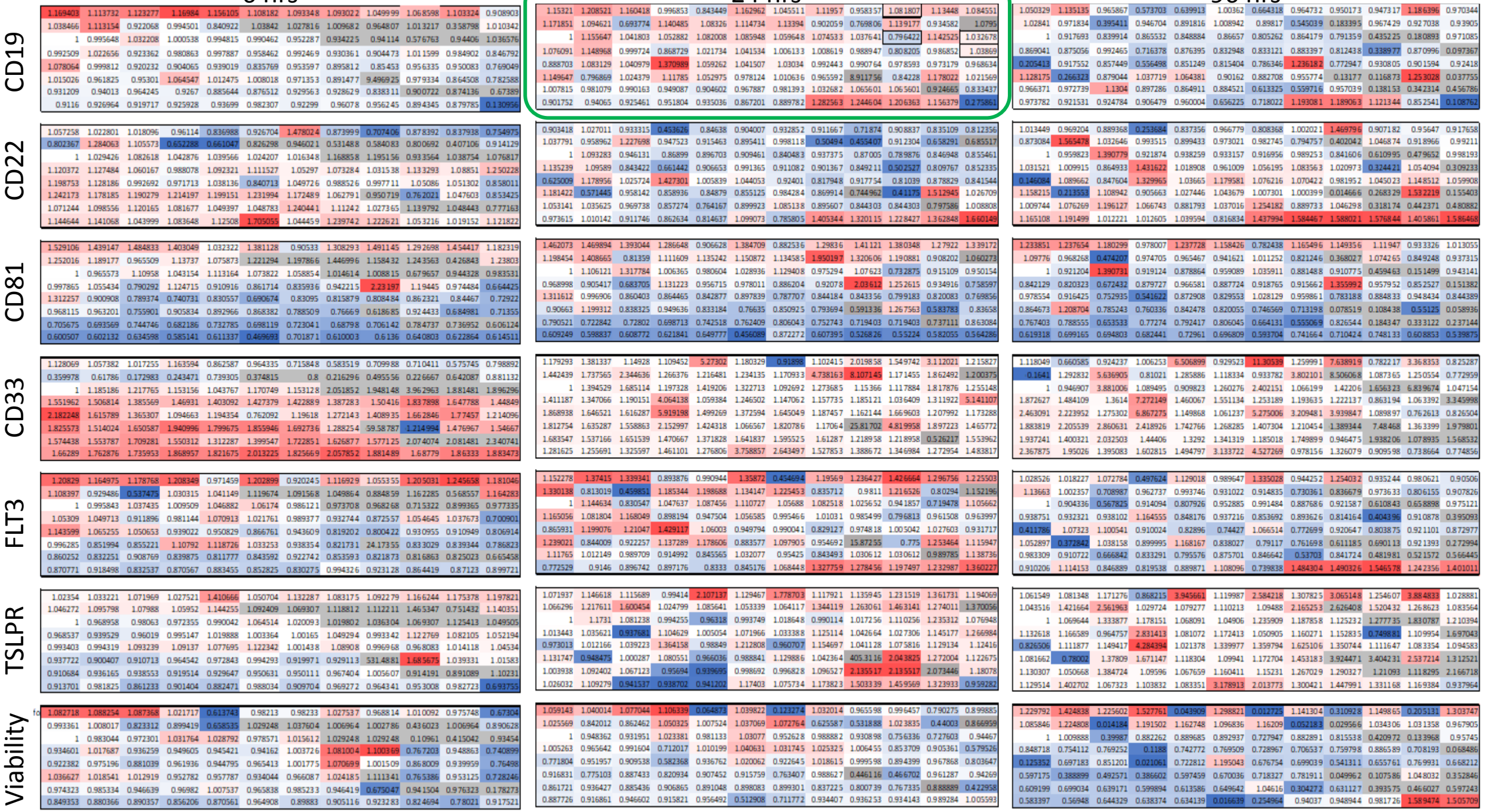
- Screen with flow cytometry
- Markers
  - CD19
  - CD22
  - CD33
  - FLT3
  - TSLPR
  - CD81
  - Viability

CAR targets

Associated with CD19

Drugs that increase antigen expression

- Mechanism
- *In vitro* and *in vivo* CAR-T efficacy


6 hrs
24 hrs
96 hrs
CD19
CD22
CD81
CD33
FLT3
TSLPR
Viability

Results: Flow Heatmap for CD19 in REH cell line

DMSO →

24 hrs

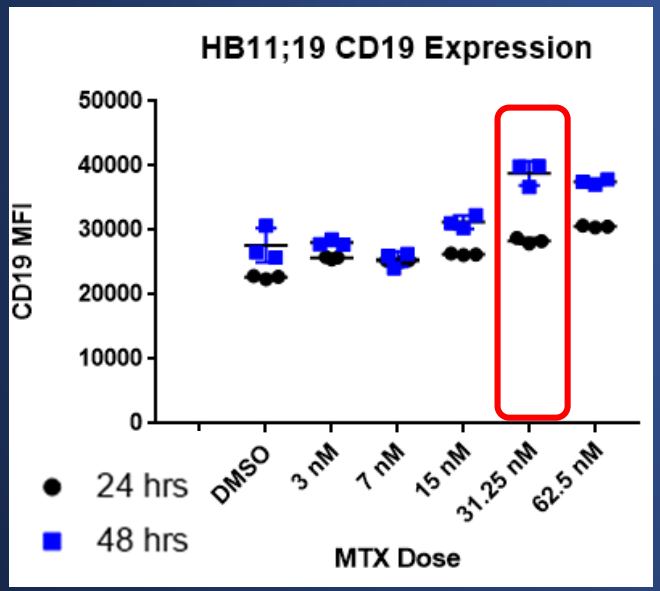
1	2	3	4	5	6	7	8	9	10	11	12
1.15321	1.208521	1.160418	0.996853	0.843449	1.162962	1.045511	1.11957	0.958357	1.081807	1.13448	1.084551
1.171851	1.094621	0.693774	1.140485	1.08326	1.114734	1.13394	0.902059	0.769806	1.139177	0.934582	1.0795
1	1.155647	1.041803	1.052882	1.082008	1.085948	1.059648	1.074533	1.037641	0.796422	1.142525	1.032678
1.076091	1.148968	0.999724	0.868729	1.021734	1.041534	1.006133	1.008619	0.988947	0.808205	0.986852	1.03869
0.888703	1.083129	1.040979	1.370989	1.059262	1.041507	1.03034	0.992443	0.990764	0.978593	0.973179	0.968634
1.149647	0.796869	1.024379	1.11785	1.052975	0.978124	1.010636	0.965592	8.911756	0.84228	1.178022	1.021569
1.007815	0.981079	0.990163	0.949087	0.904602	0.967887	0.981393	1.032682	1.065601	1.065601	0.924665	0.833437
0.901752	0.94065	0.925461	0.951804	0.935036	0.867201	0.889782	1.282563	1.244604	1.206363	1.156379	0.275861

Fold change >1

Fold change = 1

Fold change <1

<2 reps available



- MTX causes the greatest ↑ in CD19 fold change at 24 hrs in both lines
  - 1.37 fold change in REH, 1.49 in HB11;19
- Optimized dose and time (30 nM, 48 hrs)

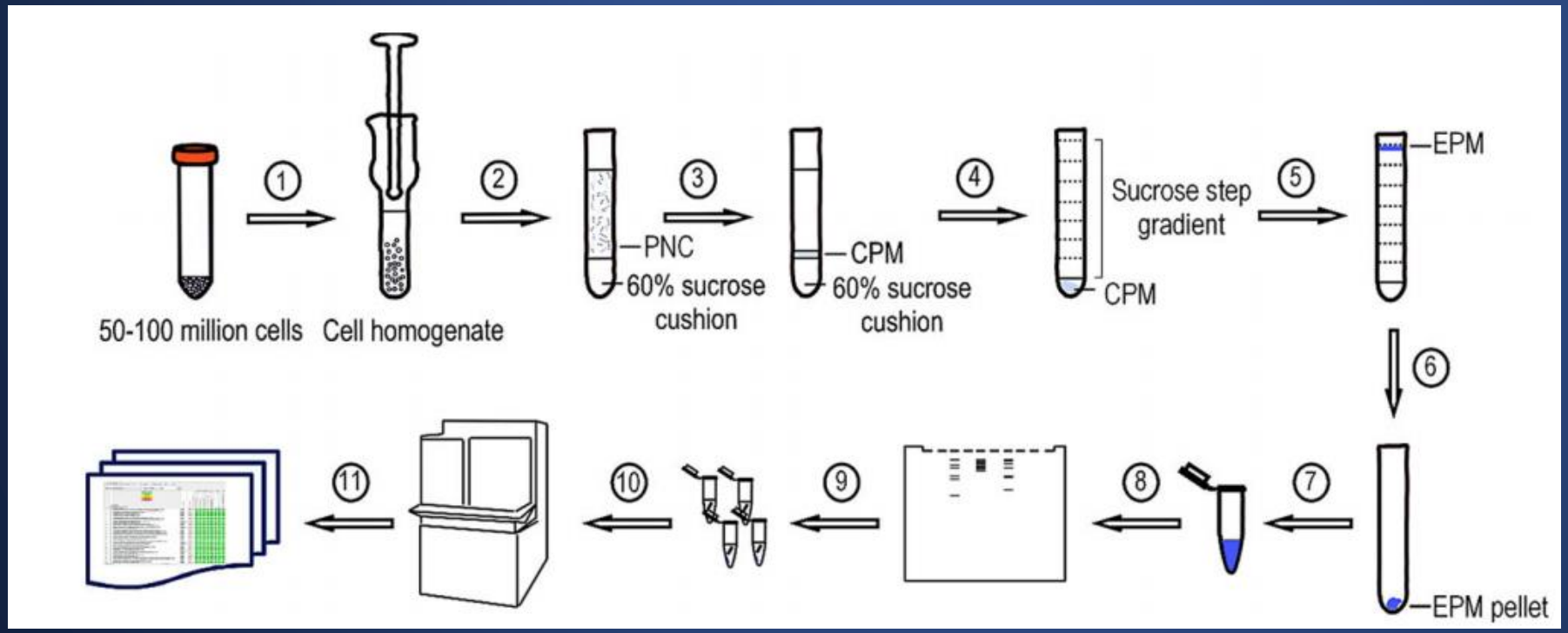
	Median (IQR) MFI Control	Median (IQR) MFI MTX 30 nM, 48 hrs	P value for Control vs. MTX
REH	**6967** (5919, 7260)	**10058** (8280, 13582)	P = 0.008
HB11;19	**27993** (27305, 35425)	**53448** (50772, 57764)	P = 0.016

MTX = methotrexate

MFI = geometric mean fluorescence intensity

Can we identify new antigenic targets?

- Defining the proteins that compose the cellular membrane remains challenging
  - Low abundance
  - Hydrophobic/lipid characteristics
  - Low membrane to cytoplasm ratios
- Gene expression profiling has not allowed us to draw reliable conclusions about the events at the protein level given the poor correlation between mRNA and protein expression



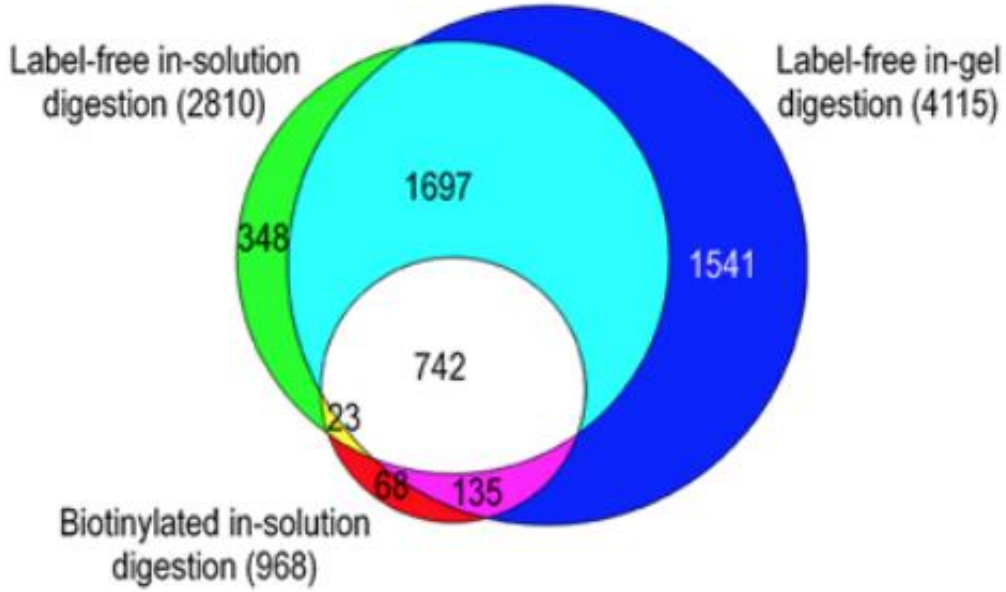
Development of a robust, label-free, nonaffinity-purified MS workflow

Proteomics. 2017 Apr;17(7).

Can we identify new antigenic targets?

- Human surfaceome is predicted to contain around 3700 proteins based on in silico prediction
- Using standard procedures, no MS study has reported more than 870 PM associated proteins
- Identified 4115 proteins
- 1938 (47%) were previously annotated as plasma membrane

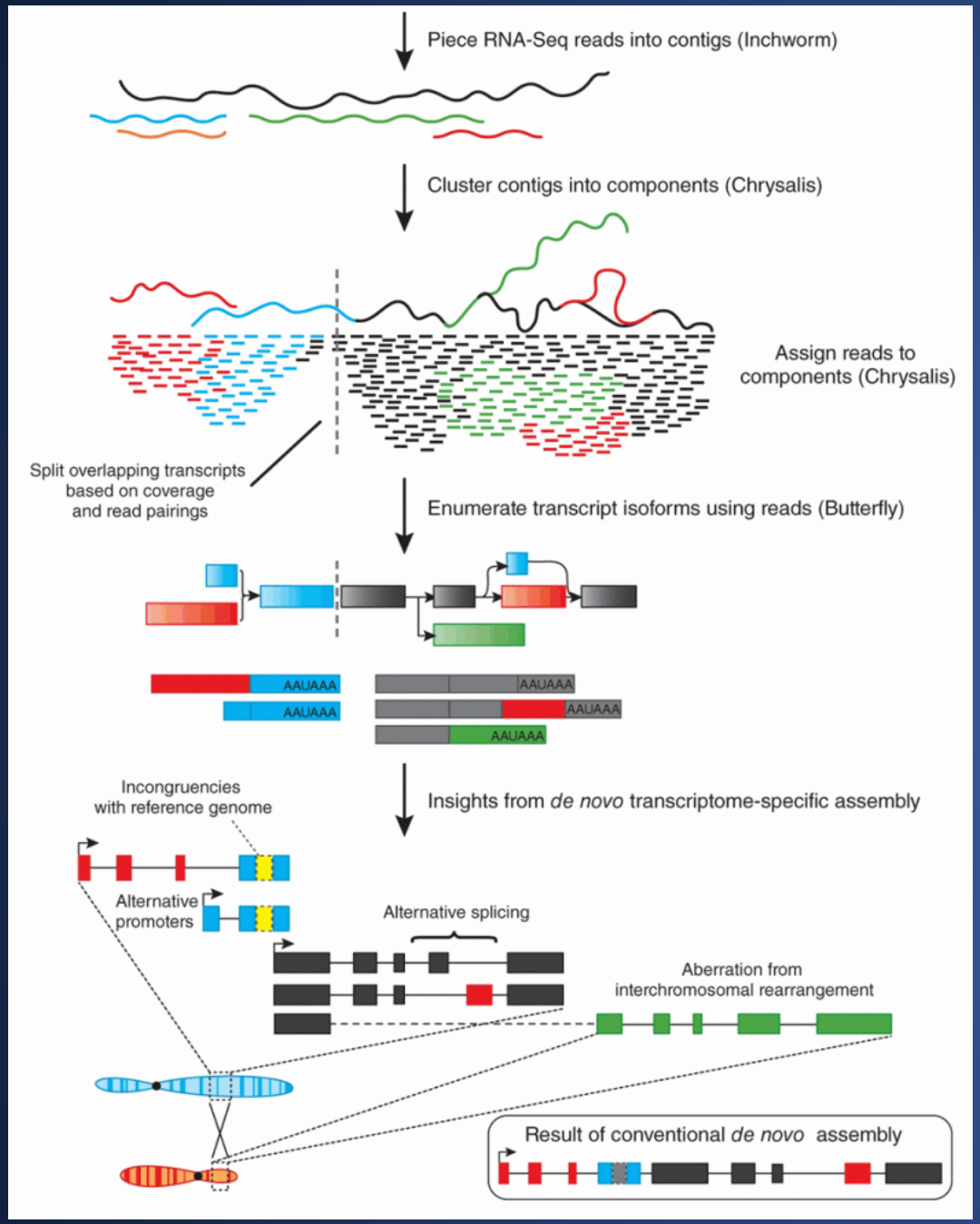
A



B

	Biotin labeled	Label-free in solution	Label-free in-gel
Protein groups	968	2810	4115
Total peptide IDs	3043	14112	37160
Surfaceome proteins	636	1223	1938
Relative enrichment %	66%	44%	47%

Proteomics. 2017 Apr;17(7).



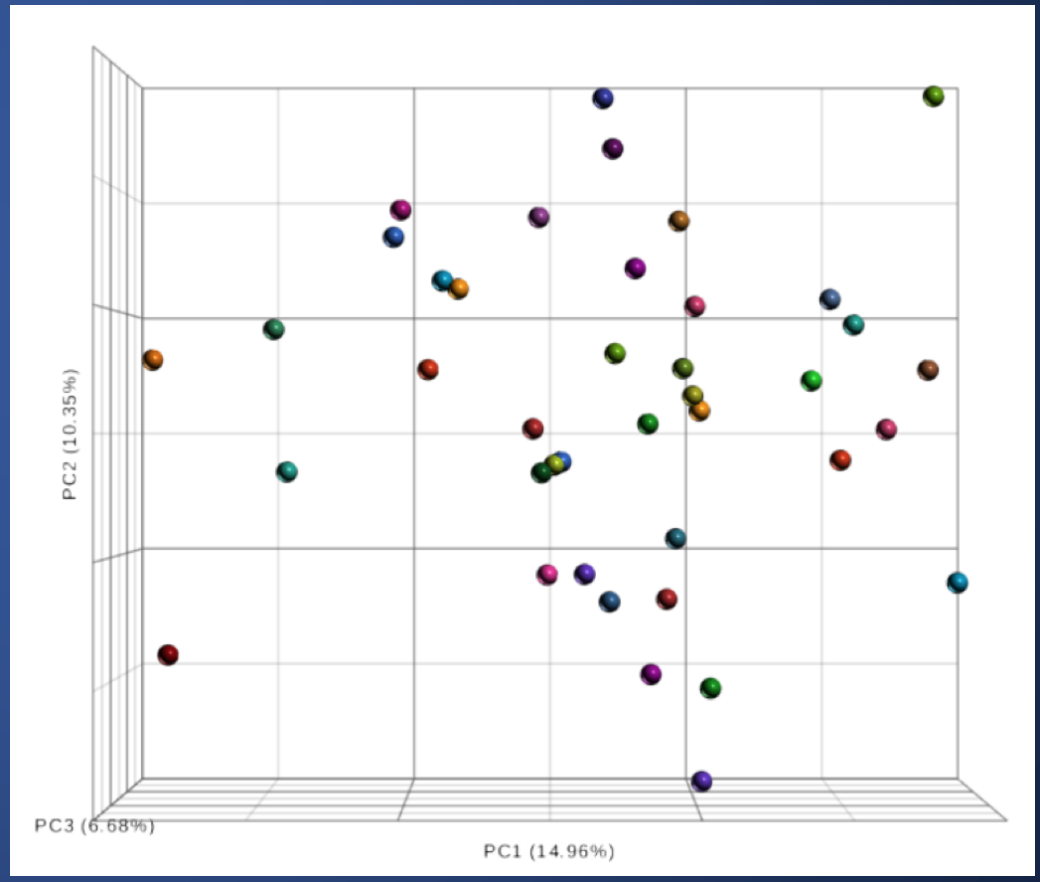
Goal: generate a sample specific "reference transcriptome"

Assembled reference transcriptome → Map MS data

- Map to known reference transcriptome - Ensembl
- De novo assembly of RNAseq data

High coverage ribosome depleted RNAseq of 6 AML cell lines

40 AML patient samples data mapped and de novo assembly completed



Nat Protoc. 2013 Aug;8(8):1494-512.

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